For example, we can utilize this X-ray resultant force for the trapping of nanoprobe and the crystal growth azimuth control during crystal growths. In addition, we proved that the ultra-fast DXT using protein molecules labeled the gold nanocrystal [2] can detect aN level's force field in functional protein molecules. In the future, we can detect dynamic structural changes of functional surface induced by an ultra-small force field that cannot be detected by STM and AFM.

[1] Y. C. Sasaki et al., Appl. Phys. Lett., 89, 053121(2006).
[2] H. Shimizu et al., Cell, 132, 67–78 (2008).

Cryo Electron Microscopy & Reconstruction

1996-Pos Board B766

Three-Dimensional Ultrastructure of Micrometer Thick Cellular Subvolumes by STEM Tomography

Alioscka A. Sousa[1], Christine A. Winters[2], Daniel Cox[1], Xiaobing Chen[2], Thomas S. Reese[2], **Richard D. Leapman**[1].
[1]NIBIB, National Institutes of Health, Bethesda, MD, USA, [2]NINDS, National Institutes of Health, Bethesda, MD, USA.

Electron tomography (ET) is a powerful technique for determining 3D ultrastructure at the supramolecular scale in sections of rapidly frozen, freeze-substituted, stained, embedded and sectioned cells and tissues. The application of conventional ET is limited to sections of thickness less than about 400 nm due to image blurring of multiply scattered electrons that are affected by the chromatic aberration of the objective lens. Many important structures such as pre- and post-synaptic nerve terminals in brain have larger dimensions so cannot be imaged in their entirety by conventional ET methods. We have therefore developed and applied tomography based on the scanning transmission electron microscope (STEM) operating at a beam energy of 300 keV. In STEM, the absence of imaging lenses after the specimen enables ET to be performed on plastic sections up to 1.5 micrometers in thickness without the deleterious effect of chromatic aberration on electrons that have undergone multiple inelastic scattering. Furthermore, it was found that the spatial resolution of 3D reconstructions obtained with an axial bright-field STEM detector was greatly improved relative to that obtained with a standard annular dark-field STEM detector, for regions in the lower half of micrometer thick sections. This advantage is attributable to the exclusion of electrons that have undergone multiple elastic scattering from the collection angles subtended by the axial bright-field detector. We have applied STEM tomography to image entire postsynaptic densities (PSDs) in dissociated cultures of rat hippocampus, in which specific proteins had been knocked down by RNAi. The technique allows multiple PSDs to be reconstructed from contiguous regions of neurons. The 3D ultrastructure reveals the role of key scaffolding proteins in the organization of PSDs.

This work was supported by the Intramural Research Programs of NIBIB and NINDS, NIH.

1997-Pos Board B767

Cryo-Electron Microscopy (Cryo-EM) Structure of a Cap-Independent Translational Enhancer of the Turnip Crinkle Virus (TCV) Bound to the Eukaryotic Ribosome

Gyanesh Sharma[1], Vera Stupina[2], Jesper Pallesen[1], Bruce Shapiro[3], Anne Simon[2], Jonathan Dinman[2], Joachim Frank[1].
[1]Columbia University, New York, NY, USA, [2]University of Maryland, College Park, MD, USA, [3]National Cancer Institute, Frederick, MD, USA.

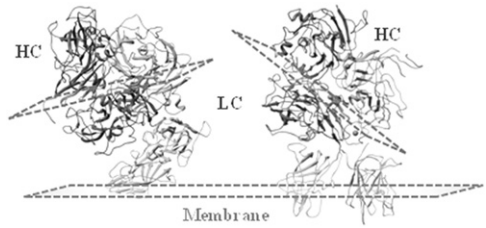
Many plant viruses, unlike animal viruses, use 3′ translational enhancers that function by unknown mechanism to achieve translation initiation. A translational enhancer in the 3′-UTR of turnip crinkle virus (TCV) has been shown to synergistically enhance translation when associated with the TCV 5′-UTR. The major enhancement has been shown, biochemically, due to a T-shaped structure (TSS) which binds to the 80S ribosomes with the aid of a pseudoknot that mimics the tRNA acceptor stem. The research presented here focuses on the structural interaction of the TSS structure with the 80S eukaryotic ribosome from Saccharomyces cerevisiae using the single-particle cryo-EM reconstruction technique. Our preliminary work shows that the density corresponding to the TSS structure is directly visible in the cryo-EM map, and most of the ribosomes containing the TSS structure are in the ratcheted-like intersubunit-rotated state. These results match the biochemical results. We are currently investigating the detailed binding interactions between the TSS and the ribosome. Interpretation of this cryo-EM map is a first step toward an understanding of the molecular mechanism of translation enhancement and regulation by the TSS structure.

1998-Pos Board B768

Factor VIII Membrane-Bound Organization

Matthew C. Leitch, Jaimy Miller, Kirill Grushin, Svetla Stoilova-McPhie.
UTMB, Galveston, TX, USA.

Factor VIII (FVIII) is a large plasma glycoprotein, which defects or deficiency cause Hemophilia A. Its active form, FVIIIa act as a co-factor to the serine protease Factor IXa within the membrane-bound tenase complex, enhancing FIXa catalytic efficiency > 100,000 fold. Understanding the assembly and structure of the tenase complex is critical for the treatment of blood disorders such as hemophilia and thrombosis. To this goal we design suitable lipid nano-platforms allowing extensive structure-functional studies required for successful nano-drug design. Here we present the membrane-bound models of two recombinant FVIII (Forms): human - full length (hFVIII-FL) and porcine - B domain deleted (pFVIII-BDD), based on our Cryo-electron microscopy and molecular modeling analysis.



Models of membrane-bound FVIII based on electron microscopy (left) and X-ray (right) structures. The dashed line rectangles delineate the heavy chain (HC) - light chain (LC) and LC - membrane interfaces, respectively.

*This work is supported by an American Heart Association: Grant, 10SDG3500034.

1999-Pos Board B769

Structural Investigation of Molecular Components of T. Brucei Flagellum by Cryo Electron Tomography

Alexey Y. Koyfman[1], Michael F. Schmid[1], Ladan Gheiratmand[2], Caroline J. Fu[1], Dandan Huang[2], Htet A. Khant[1], Cynthia Y. He[2], Wah Chiu[1].
[1]Baylor College of Medicine, Houston, TX, USA, [2]National University of Singapore, Singapore, Singapore.

Trypanosoma brucei is a parasite that causes trypanosomiasis or African sleeping sickness. The Trypanosoma brucei contains a flagellum that is vital for the organism's locomotion, pathogenesis, cell division and viability. In addition to a 9+2 microtubular axoneme, the flagellum contains a crystalline paraflagellar rod (PFR) and connecting proteins bridging these two structures. We have recently reported the structure of T. brucei flagellum by cryoelectron tomography and proposed that the PFR modifies the in-plane axoneme motion to produce the characteristic trypanosome bihelical motility. We have improved the flagellum extraction procedure by reducing salt concentration. The modified procedure kept flagellum shielded is the lipid membrane and therefore preserved molecular components of the flagellum. We also revealed a characteristic arrangement of the axoneme internal features. An arrangement of three radial spoke repeat along a microtubule doublet. Also, the arrangement of radial spoke triplets on the nine microtubule doublets is not found in other organisms.

2000-Pos Board B770

Subunit Organization of the 26S Proteasome and Structural Basis for Processing of Ubiquitin-Tagged Substrates

Gabriel C. Lander[1], Eric Estrin[2], Mary Matyskiela[2], Eva Nogales[1], Andreas Martin[2].
[1]Lawrence Berkeley National Lab, Berkeley, CA, USA, [2]UC Berkeley, Berkeley, CA, USA.

In eukaryotic cells, the proteasome degrades unwanted proteins by recognizing specific polyubiquitin tags covalently attached to these proteins. The precise manner in which these ubiquitin chains are recognized and removed from the targeted proteins prior to proteolysis is poorly understood. This is partly due to a lack of structural information on the ubiquitin-recognizing components of the proteasome 19S regulatory particle. Using a recombinant expression system and electron microscopy, we were able to localize all subunits of the yeast 19S particle, and elucidate the spatial arrangement of ubiquitin receptors, deubiquitinating enzymes, and the protein unfolding machinery. Our studies also revealed large conformational rearrangements in the lid subcomplex upon holoenzyme formation, suggesting an allosteric mechanism for activation